

Short Communication

The selection of buffer salts for the high-performance liquid chromatography of basic compounds

BRIAN LAW* and PAN F. CHAN

Drug Kinetics Group, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

Keywords: HPLC; eluents; buffers; basic drugs; acetate salts.

Introduction

The use of silica with reversed-phase type eluents at alkaline pH offers a useful approach to the analysis of basic compounds by high-performance liquid chromatography (HPLC). The original buffer salt was ammonium nitrate [1], which has been used widely, and for which considerable retention data are available [2, 3]. Whilst being considered a volatile salt and a good buffer at alkaline pH, the use of ammonium nitrate gives one major draw-back. The high ultraviolet (UV) absorbance of the nitrate anion at low wavelengths limits the use of such a system to detection at >250 nm, which can be a serious limitation in drug analysis.

The obvious replacement anions; sulphate and chloride (both of which are strong acids) give non-volatile ammonium salts — volatility being a useful feature in preparative HPLC and combined HPLC mass spectrometry (LC-MS) — and are not fully UV transparent. Chloride also has the disadvantage of dissolving stainless steel. The other common HPLC buffer anion, phosphate does not give soluble ammonium salts in aqueous methanol solutions.

Turning to the organic acids; citric, acetic and trifluoroacetic which are commonly used as HPLC eluent constituents, only trifluoroacetic and acetic acid give volatile ammonium salts.

The present work evaluates and compares the use of ammonium acetate, and trifluoroacetate (both of which are widely used in

HPLC) with nitrate in terms of their physical, UV, buffering and electrochemical properties as well as their effect on chromatographic performance.

Experimental

Equipment

Ultraviolet spectra were recorded using a Pye SP8000 spectrophotometer, pH measurements were made using a Beckman 3500 digital pH meter equipped with a CMAWL combined pH glass/reference cell (Russell pH Ltd, Fife, UK). The pH electrode incorporated a ceramic frit liquid junction and an Ag/AgCl reference electrode with 3 M potassium chloride as the reference electrolyte. The system was calibrated with aqueous buffers. Electrochemical measurements were carried out using either a static platinum electrode or a rotating glassy carbon electrode coupled to a Wenking LB75H potentiostat and a Princeton Applied Research 175 waveform generator. HPLC was carried out using a Waters 6000A pump, a Rheodyne 7125 injection valve and an LDC UV-III fixed wavelength (254 nm) detector.

Materials

Methanol and acetonitrile were HPLC grade, nitric acid (70%, w/w), acetic acid, ammonia (25%, w/w), ammonium acetate and ammonium nitrate were Analar grade. Ammonium trifluoroacetate (98%) was from Aldrich, trifluoroacetic acid was either high purity grade (99+%) from Aldrich and Pierce, or standard grade (99%) from Aldrich. The

* Author to whom correspondence should be addressed.

two high purity grades were recommended for protein sequencing due to their low UV background.

The standard HPLC eluent was methanol-water-ammonium nitrate buffer (9:0.5:0.5, v/v/v), with a final pH of 9.1. The buffer was prepared from ammonia (65 ml), nitric acid (11 ml) and water (424 ml). Acetate and trifluoroacetate buffers were prepared with an equivalent molar concentration of the appropriate acid. The concentration of ammonia in the buffer was 1.74 M and the acid 0.352 M.

Methods

Buffers were diluted in water, and the UV spectrum measured over the range 190–450 nm. For titration experiments buffers were diluted (1 + 9) in water or methanol and the buffering capacity measured by titration against aqueous hydrochloric acid or sodium hydroxide (both 2 M).

To assess the volatility of various salts, 20 mg of finely ground ammonium acetate, nitrate and trifluoroacetate were placed under vacuum at room temperature. The appearance, vapour pressure and weight loss were recorded over 36 h. The solubility of the salts was also measured in water, methanol and acetonitrile.

The background current generated by eluents prepared from the three buffers were studied by cyclic voltammetry using a platinum electrode, and under pseudo-steady-state conditions using a rotating glassy carbon electrode. Measurements were made over the potential range 0 to +1.1 V.

The effect of buffer anion on chromatographic selectivity was examined using a 100 × 4.6 mm i.d. column packed with Spherisorb S5W silica and two eluents employing the nitrate and acetate anions at the same molar concentrations. The test solutes consisted of phenelzine, orphenadrine, propranolol, phenformin, protriptyline, chloroquine and benz-

ethonium chloride. A further set of test compounds was also used, consisting of the anti-malarial drugs proguanil and chlorproguanil along with their cyclo and desalkyl metabolites.

Results and Discussion

UV properties

The UV absorbance for solutions of various ammonium salts at selected wavelengths is given in Table 1. It is surprising, given the very high absorbance of the nitrate anion right across the spectral range and particularly at low wavelength (<250 nm) that nitrate was ever selected as an eluent component. In contrast, acetate and trifluoroacetate show similar absorbance, which only becomes significant at <215 nm. The high purity grades of trifluoroacetic acid show little advantage over the standard grade, despite the claims of the manufacturers.

Buffering capacity

The buffering capacity of ammonium acetate, both in water and methanol-water (9:1, v/v), is shown in Fig. 1, as a variation in pH against the volume of sodium hydroxide and hydrochloric acid added, 2 M in each case. The buffering capacity of the aqueous solution is somewhat superior to that of the organic-aqueous mixture, however, both solutions show reasonable buffering capacity over the pH range 8–9.5.

The buffering properties of ammonium nitrate and ammonium trifluoroacetate (data not shown) were also very similar to that of ammonium acetate, and in this respect, there was little to choose between the three.

Good buffering capacity is obviously important in ensuring good chromatographic reproducibility and on the basis of the above data, any of the three salts would be satisfactory.

Table 1
Absorbance of buffers at different wavelengths

Ammonium buffer anion*	Absorbance at various wavelengths (nm)						
	200	210	220	230	240	250	300
NO ₃ ⁻	136	>140	139	38.1	4.75	0.150	0.258
CH ₃ CO ₂ ⁻	2.61	1.98	0.517	0.103	0.010	0.001	0.0
CF ₃ CO ₂ ⁻ (standard)	2.65	1.70	0.569	0.191	0.054	0.013	0.0
CF ₃ CO ₂ ⁻ (Aldrich 99% +)	2.62	1.80	0.590	0.194	0.059	0.020	0.0
CF ₃ CO ₂ ⁻ (Pierce 99.5%)	2.64	1.70	0.560	0.183	0.056	0.014	0.0

* All buffers were of the same molarity (0.035 M) with respect to the anion.

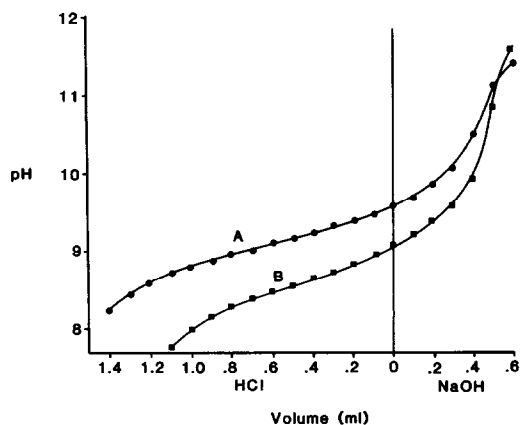


Figure 1
Titration curves for ammonium acetate buffer in water (A) and in methanol-water (9:1, v/v) (B).

Physical properties

A search of the literature failed to provide any data on the volatility of the ammonium salts of interest, with the exception of ammonium nitrate which was reported as decomposing at 210°C [4]. There was also only limited data [4, 5], on the solubility of these compounds in common HPLC solvents.

The work carried out here showed ammonium acetate to be much more volatile than either of the other two salts. At room temperature, ammonium acetate had a vapour pressure of approximately 7.2 mbar, and 20 mg was completely volatilized within 60 min. In contrast neither the nitrate or trifluoroacetate salt showed significant volatilization after 36 h (vapour pressure <1.2 mbar). The high volatility of ammonium acetate obviously favours its use in preparative HPLC and in combined LC-MS.

The approximate solubility for the three salts in the common HPLC solvents measured at room temperature (25°C) is shown in Table 2. The solubility properties of ammonium trifluoroacetate was the best in all three solvents, and in the case of acetonitrile very much better

than that of the acetate or nitrate salts. Given the very high solubility of all three salts in water, it is unlikely that solubility will have any practical consequence except for eluents containing a very high proportion of acetonitrile.

Electrochemical properties

In HPLC with oxidative electrochemical detection, the background current generated by the eluent constituents frequently limits the successful application of the technique. Measurements were therefore made on the eluents in question to assess any differences in background current. In the cyclic voltametry studies with the platinum electrode the three eluents showed similar patterns with background current increasing exponentially with increasing voltage, reaching $>140 \mu\text{A cm}^{-1}$ at +1.0 V.

Under pseudo-steady-state conditions the voltamograms were relatively flat up to 0.8 V, from where they rose steeply. At a potential of 1.1 V the trifluoroacetate eluent showed a slightly lower background current, $40 \mu\text{A cm}^{-1}$ compared with $55 \mu\text{A cm}^{-1}$ for the other two eluents. However, given the small difference in background current, coupled with the steep rise in the voltamogram at this point, there is probably no practical difference in the electrochemical properties of the eluents under the conditions studied.

Chromatographic performance

Schmid and Wolf [6] have reported selectivity changes in the chromatography of bases on silica with variations in the buffer anion. It was considered essential therefore to compare retention data generated using ammonium nitrate and what appeared to be the buffer of choice, ammonium acetate.

The data in Table 3 show good agreement between the selectivity values (α) and capacity factors (k') for the two systems. The major exception to this was the closely eluted anti-

Table 2
Approximate solubility data for the ammonium salts of nitric, acetic and trifluoroacetic acids in common HPLC solvents at room temperature

Salt	Approximate solubility (g/100 ml)		
	Water	Methanol	Acetonitrile
Ammonium nitrate	200*	14	<0.2
Ammonium acetate	148†	55	<0.2
Ammonium trifluoroacetate	260	>80	5.2

*From ref. 4.

†From ref 5 measured at 4°C.

Table 3
Capacity factors (k') and selectivity values (α) for critical pairs using eluents prepared from ammonium nitrate and ammonium acetate

Compound	Nitrate eluent		Acetate eluent	
	k'	α	k'	α
Phenelzine	0.29		0.28	
Orphenadrine	0.63	2.17	0.64	2.28
Propranolol	0.85	1.34	0.81	1.27
Phenformin	2.31		2.17	
Protriptyline	3.61		3.36	
Chloroquine	8.06		8.11	
Benzethonium	8.17	1.01	8.44	1.04
Chloroproguanil	2.13		1.89	
Proguanil	2.22	1.04	1.83	0.97
Desalkylchloroproguanil	2.56	1.15	2.25	1.23
Desalkylproguanil	2.62	1.02	2.33	1.04
Chlorcycloguanil	3.16	1.21	2.80	1.20
Cycloguanil	3.35	1.06	2.98	1.06

malarials proguanil and chloroproguanil where the order of elution was reversed on changing the eluent. These type of compounds have been previously shown to be sensitive indicators of column selectivity [7], and are generally atypical in this respect. In other respects the chromatographic performance was the same with both eluents.

Conclusions

In terms of buffering capacity and electrochemical characteristics there is little to choose between the ammonium salts of nitric, acetic or trifluoroacetate acid as eluent buffer salts for the analysis of bases on silica. In terms of UV properties, volatility and cost, however, ammonium acetate is superior. Ammonium trifluoroacetate has a small advantage in terms of solubility in the common HPLC solvents although this is not considered significant. The change from nitrate to acetate which is strongly recommended here would appear to have only minor effects on chromatographic selectivity. Whilst this work was in progress, Smith and Rabour [8] reported on the use of the cationic buffer constituent ethylenediamine for the analysis of basic drugs on silica and recommended this in place of ammonia. The anionic component of the eluent (nitrate) was not studied.

The preference for ammonium acetate here ties in well with the current widespread use of

this buffer in LC-MS systems employing the thermospray interface. Its use in LC-MS is based on good solubility and high volatility. Although the silanol masking properties of ammonia are undoubtedly inferior to alkylamines, ammonium acetate has also been recommended as a general purpose buffer for the reversed-phase HPLC of bases [9]. The data generated here further support this recommendation.

Acknowledgements — We wish to thank S. Forsythe of ICI Chemicals and Polymers Limited (Runcorn, Cheshire) for carrying out the electrochemical measurements and P. Clucas for typing the manuscript.

References

- [1] I. Jane, *J. Chromatogr.* **111**, 227–233 (1975).
- [2] B. Law, R. Gill and A.C. Moffatt, *J. Chromatogr.* **301**, 165–172 (1984).
- [3] B. Law, *J. Chromatogr.* **407**, 1–18 (1987).
- [4] *Merck Index*, 10th edn. Merck & Co., Rathway, NJ (1983).
- [5] *Handbook of Chemistry and Physics*, 69th edn. CRC Press, Florida (1988–1989).
- [6] R.W. Schmid and C.H. Wolf, *Chromatographia* **24**, 713–719 (1987).
- [7] B. Law and P.F. Chan, *J. Chromatogr.* **467**, 267–271 (1989).
- [8] R.M. Smith and J.O. Rabour, *J. Chromatogr.* **464**, 117–123 (1989).
- [9] C.K. Lim and T.J. Peters, *J. Chromatogr.* **316**, 397–406 (1984).

[Received for review 14 June 1990;
revised manuscript received 4 December 1990]